Amendments to the Specification

Please amend the paragraph beginning at page 3, line 6 as follows.

In a first embodiment, the invention provides an isolated polynucleotide having at least 70% sequence identity with the nucleotide sequence, SEQ ID No.:1 shown in Figure ~~1~~ 1, and having pin1 gene promoter activity.

Please amend the paragraph beginning at page 3, line 10, as follows.

In another embodiment, the invention provides an isolated DNA sequence comprising a polynucleotide molecule selected from the group consisting of that shown in Figure 1 (SEQ ID No.:1), Figure 2 (SEQ ID No.:2), Figure 3 (SEQ ID No.:3), and any functional elements thereof having pin1 promoter activity.

Please amend the paragraph beginning at page 3, line 14, as follows.

In another embodiment, the invention provides an isolated polynucleotide having at least 70% sequence identity with the nucleotide sequence shown in Figure 4 (SEQ ID No.:4), and having amt gene promoter activity.

Please amend the paragraph beginning at page 3, line 17, as follows.

In another embodiment, the invention provides an isolated DNA sequence comprising a polynucleotide molecule selected from the group consisting of that shown in Figure 4 (SEQ ID No.:4), Figure 5 (SEQ ID No.:5), and functional elements thereof having pin1 promoter activity.

Please amend the paragraph beginning at page 3, line 20, as follows.

In another embodiment, the invention provides a cDNA molecule having the nucleotide sequence shown in Figure 8 (SEQ ID No.:6) which corresponds to the pin1 ~~amt1~~ gene.

Please amend the paragraph beginning at page 3, line 22, as follows.

In yet another embodiment, the invention provides a cDNA molecule having the nucleotide sequence shown in Figure 9 (SEQ ID No.:7) which corresponds to the amt gene.

Please amend the paragraph beginning at page 4, line 14, as follows.

Fig. 5. illustrates the DNA sequence of isoform II ~~1~~ from amt gene promoter (SEQ ID NO:5) highlighting the translation start site, TATA box and CAATT box.

Please amend the paragraph beginning at page 4, line 23, as follows.

Fig. 8. illustrates the DNA sequence (SEQ ID No.:6) of the cDNA corresponding to the pin1 gene.

Please amend the paragraph beginning at page 4, line 25, as follows.

Fig. 9. illustrates the DNA sequence (SEQ ID No.:7) of the cDNA corresponding to the amt gene.

Please amend the paragraph beginning at page 12, line 24, as follows.

One type of embodied promoter of the present invention is the proteinase inhibitor (pin1) promoter. There are provided four isoforms of the pin1 promoter. The DNA

sequence of isoform I (SEQ ID No.:1), isoform II (SEQ ID No.:2), and isoform III (SEQ ID No.:3) of the pin1 promoter is disclosed as Figures 1, 2, and 3, respectively.

Please amend the paragraph beginning at page 12 line 28, as follows.

Referring to Fig. 1, for example, the full-length sequence of the pin1 gene promoter isoform I (SEQ ID No.:1) is shown. The ~~shaded area 100 represents~~ the highlighted areas represent the translation start site (ATG), ~~(ATG). The underlined area 102 represents~~ the TATA box, and ~~the underlined area 104 represents~~ the CAATT box.

Please amend the paragraph beginning at page 13, line 1, as follows.

The Eukaryotic Promoter Database ~~(http://srs.ebi.ac.uk:9999/srs6bin/cgi-bin/wgetz?-page+LibInfo+-id+4Flds1EqUDE+-lib+EPD)~~ was searched. None of the isoforms of the pin1 gene promoter of this invention exhibits significant sequence homology compared to any eukaryotic promoters reported to date and verified using homology search methods such as BLAST. The highest matching sequence found is gnl|EPD|14007(+)Pv[dlec2] PHA-L, with an E value of 0.001, which is not significantly homologous to the promoter isoform sequence of this invention.

Please amend the paragraph beginning at page 13, line 8, as follows.

In one embodiment, the promoter of this invention comprises the entire sequence of isoform I of the pin1 gene promoter (SEQ ID No.:1), and the various functional segments thereof. ~~In another embodiment, the promoter of this invention comprises the entire sequence of isoform Iof the pin1 gene promoter, and the various functional segments thereof~~. In another embodiment, the promoter of this invention comprises the entire sequence of isoform II of the pin1 gene promoter (SEQ ID No.:2), and the various functional segments thereof. In yet ~~anther~~ another embodiment, the promoter of this invention comprises the entire sequence of isoform III of ~~IIIof~~ the pin1 gene promoter (SEQ ID No.:3), and the various functional segments thereof. In another embodiment, the polynucleotide of the invention has at least 70%, more preferably 80%, most preferably 90%, sequence identity with any one of SEQ ID Nos.:1-3 ~~isoforms I, II, and III~~ and has pin1 gene promoter activity.

Please amend the paragraph beginning at page 14, line 10, as follows.

Another type of embodied promoter of the present invention is the aminotransferase (amt) gene promoter. There are provided two isoforms of the amt gene promoter. The full length sequence of isoform I (SEQ ID No.:4), and isoform II (SEQ ID No.:5), of the amt gene promoter is disclosed in Figures 4 and 5, respectively.

Please amend the paragraph beginning at page 14, line 14, as follows.

Referring to Fig. 4, for example, the full-length sequence of the amt gene promoter isoform I (SEQ ID No.:4) is shown. The shaded ~~area 200 represents~~ (highlighted) areas represent the translation start site (ATG), ~~(ATG). The underlined area 202 represents~~ the TATA box, and ~~the underlined area 204 represents~~ the CAATT box.

Please amend the paragraph beginning at page 14, line 18, as follows.

Neither isoform I nor isoform II of the amt gene promoter of this invention shares any significant sequence homology with any eukaryotic promoters reported to date. Sequence homology searches were performed, for example a BLAST search, using the collection of the promoter sequences in the Eukaryotic Promoter Database ~~(http://srs.ebi.ac.uk:9999/srs6bin/cgi-bin/wgetz?-page+LibInfo+-id+4Flds1EqUDE+-lib+EPD~~). The highest matching sequence found is gnl|EPD|11005 (+) Am chalcone synthase, with an E value of 0.17, which is not significantly homologous to this promoter isoform sequence.

Please amend the paragraph beginning at page 14, line 26, as follows.

In one embodiment, the promoter of this invention comprises the entire sequence of isoform I of the amt gene promoter (SEQ ID No.:4), and the various functional segments thereof. In another embodiment, the promoter of this invention comprises the entire sequence of isoform II of the amt gene promoter (SEQ ID No.:5), and the various functional segments thereof.

Please amend the paragraph beginning at page 23, line 6, as follows.

A cDNA library was constructed from poly(A) RNA isolated from dark-treated excised potato leaf tissues using the ZAP cDNA synthesis kit (Strategene). Approximately 60,000 plaques from the cDNA library were plated, transferred onto duplicate nitrocellulose filters, hybridized ~~hibridized~~ with radiolabelled cDNA probes synthesized independently from 1 µg poly (A) RNA of healthy untreated and dark-treated leaf tissues. Plaques showing contrasting signal intensity between untreated and dark-treated probing were collected, re-plated, and rescreened using newly synthesized cDNA probes. A single, pure plaque from each of these clones still demonstrating a differential hybridization signal intensity was collected, and the pBluescript phagemid containing the cDNA insert was excised from the mUniZAP vector as described by the manufacturer (Stratagene). Nucleotide sequences were determined by automated sequencing. Homology-based searches of the GENBANK™ (United States Department of Health and Human Services,

Rockville Pike, MD) ~~Genebank~~ databases were performed using the BLASTN program. Altshul et al., J. Mol. Biol. 215: 403 (1990). The DNA sequence of the cDNA corresponding to the pin1 gene (SEQ ID No.:6) is shown in Figure 8. The DNA sequence of the cDNA corresponding to the amt gene (SEQ ID No.:7) is shown in Figure 9.

Please amend the paragraph beginning at page 23, line 24, as follows.

The promoter elements of pin1 and amt were isolated using the GENOMEWALKER™ kit (CLONTECH Laboratories, Inc., Palo Alto, CA) ~~Genome Walker™ kit (CLONETECH).~~ Briefly, potato genomic DNA was first digested with restriction endonucleases: Dra I, Eco R V, Pvu II, Sca 1, and SspI. An adapter was ligated onto the digested genomic DNA fragments to create five libraries of potato specific genomic DNA fragments corresponding to the restriction endonucleases. The genomic libraries were then used as templates in nested PCR reactions with gene-specific primers (pin1 or amt) and the adaptor primers provided from manufacturer. The PCR products were cloned into pGEM vectors for DNA fragment amplification and sequencing. The promoter elements were confirmed by comparison with known cDNA sequences since the gene-specific primers were designed about 100 to 100 bp downstream of the cDNA clone.

Please amend the paragraph beginning at page 24, line 3, as follows.

Primers used for promoter isolation are as follows:

~~Genome Walker~~ GENOMEWALKER™ Adapter primers from CLONTECH ~~CLONETECH~~

Adapter primer 1 (AP1): 5'-GTA ATA CGA CTC ACT ATA GGG C-3' (SEQ ID No.:8).

Nested adapter primer 2 (AP2): 5'-ACT ATA GGG CAC GCG TGG T-3' (SEQ ID No.:9).

Please amend the paragraph beginning at page 24, line 8, as follows.

For the proteinase inhibitor gene (pin1)

Primer SEN16 (77-51 antisense) 5-GAA AGC AAC CAA CTT CAC CAT AGA CT-3' (SEQ ID No.:10).

Primer SEN28 (65-28 antisense): 5'-CTT CAC CAT AGA CTT ATT TGC CTC CAT TTA ATT CTG CA-3' (SEQ ID No.:11).

Please amend the paragraph beginning at page 24, line 14, as follows.

For the aminotransferase gene (amt)

Primer SEN29 (119-93): 5'-CCA GCT AGA GTA TCA AGA TAC TTC CT-3' (SEQ ID No.:12).

Primer SEN30 (148-120): 5'-CGT TCC CCC CTA GTG CTG TGC ACC ACA A-3' (SEQ ID No.:13).

Primer SEN31 (178-148): 5'-GCT TAG TGG CAG CAT CAA CCA GGC GAG GCT-3' (SEQ ID No.:14).

Please amend the paragraph beginning at page 24, line 20, as follows.

The DNA sequence of the pin1 and amt genomic clones was determined by automatic sequencing of the DNA Sequencing Facility, Iowa State University, Ames, Iowa. The DNA sequences of isoforms I (SEQ ID No.:1), II (SEQ ID No.:2), and III (SEQ ID No.:3) of the pin1 gene promoter are shown in Figures 1, 2, and 3, respectively. The DNA sequences of isoform I (SEQ ID No.:4),and II (SEQ ID No.:5), of the amt gene promoter are shown in Figures 5 and 6, respectively.

Please amend the paragraph beginning at page 24, line 28, as follows.

The amt gene promoter of this invention confers light/dark sensitivity to the amt gene as reflected in the Northern analysis. Referring to Fig. 7, dark induces senescence and the enhanced expression of amt gene driven by the promoter disclosed herein. The bands 401, 402, 403, and 404 ~~301, 302, 303, and 304~~ represent the levels of expressed gene product after dark treatment of 1 day, 2 days, 3 days, and 4 days, respectively. The first lane (400) ~~(300)~~ is the control, the sample for which was taken before the dark treatment. The potato leaves were cut and maintained in the 10 mM MES buffer and treated with 100 ppm ethylene at room temperature and kept in dark. A 30ug aliquot of total RNA extracted from the treated leaves was used for electrophoresis on 1.3% of formaldehyde agarose gels. The target mRNA was fixed on the Zeta probe membrane at 65°C for 17 hrs. A ^{32}P labeled probe, a Hind III cDNA fragment of potato aminotransferase gene, was used to hybridize the membrane. After washing, x-ray film was exposed to the membranes for 2 days, and the resulting exposure for each band was measured. As revealed by Northern analysis, the pin1 gene promoter is induced by dark treatment.

Please amend the paragraph beginning at page 25, line 13, as follows.

Fig. 6 demonstrates an example of Northern analysis for expression of the pin1 gene controlled by the native pin1 gene promoter. Plants were exposed to the dark for 0, 1, 2, 3, and 4 days and gene expression monitored by Northern analysis with the results

shown in lanes 300, 301, 302, 303, and 304, ~~400, 401, 402, 403, and 404~~, respectively. The experimental protocol was similar to that described in Example 3, except that an EcoR I/Xho I restriction fragment of pin1 gene was used as the probe. As revealed by Northern analysis, the pin1 gene promoter is induced by dark treatment.

Please amend the paragraph beginning at page 27, line 19, as follows.

Referring to Figure 8, the full length cDNA sequence of pin1 gene is illustrated. Sequence homology searches were performed using the nucleic acid sequence database GENBANK™ (United States Department of Health and Human Services, Rockville Pike, MD). ~~GenBank~~. It was found that this sequence is highly homologous to the sequence of tomato fruit-ripening protein, i.e., ethylene responsive proteinase inhibitor I (er1) mRNA, with an E value of 0. This result was further confirmed by homology searches using the amino acid sequence database SWISS-PROT™ (Institut Suisse de Bioinformatique (SIB) Foundation Switzerland, Geneve Switzerland) ~~Swisprot~~. There, tomato ethylene-responsive proteinase inhibitor 1 precursor was identified as homologous with an extremely low E value (4e-59). The identity of the cDNA as encoding potato ethylene responsive proteinase inhibitor is therefore established. Northern analysis further verifies this result.

Please amend the paragraph beginning at page 27, line 31, as follows.

Figure 10 demonstrates an example of Northern analysis on the expression of pin1 gene controlled by the native pin1 promoter. Ethylene treatment for 1-24 hours at a concentration of 50 ppm was exerted and the accumulation of transcripts was measured at the time points of 1 hour ~~(Lane 702)~~, 2 hours ~~(Lane 704)~~, 4 hours ~~(Lane 706)~~, 6 hours ~~(Lane 708)~~, 10 hours ~~(Lane 710)~~, and 24 hours ~~(Lane 712)~~. Lane 0 ~~700~~ represents the zero time point; and lane 24W/O ~~Lane 714~~ represents the control where no ethylene was applied. Increased levels of expression of the gene product are observed after 4-hour treatment~~, in lanes 706, 708, 710, and 712, respectively~~. The Northern analysis was similar to that in Example 3 except that an EcoR I/Xho I restriction fragment of pin1 gene was used as the probe.

Please amend the paragraph beginning at page 28, line 26, as follows.

The present inventors identified the full length cDNA sequence of a amt gene (SEQ ID No.:7) as shown in Fig. 9. Sequence homology searches were performed using the nucleic acid sequence database GENBANK™ (United States Department of Health and Human Services, Rockville Pike, MD). ~~GenBank~~. It was shown that this sequence is highly homologous to the sequence of capsicum Chinese strain habanero putative aminotransferase mRNA, with an E value of 0. This result was further confirmed by homology searches using the amino acid sequence database SWISS-PROT™ (Institut

Suisse de Bioinformatique (SIB) Foundation Switzerland, Geneve Switzerland) ~~Swisprot~~. The aminotransferase-like protein from *Arabidopsis thaliana* was identified as homologous with an E value of 0. The identity of the cDNA as encoding potato aminotransferase is therefore established. Northern analysis was also performed with this gene.

Please amend the paragraph beginning at page 29, line 6, as follows.

Figure 11 is an example of Northern analysis on the expression of the amt gene controlled by the native amt gene promoter. Ethylene treatment for 1-24 hours at a concentration of 50 ppm was exerted and the accumulation of transcripts was measured at the time points of 1 hour ~~(Lane 802)~~, 2 hours ~~(Lane 804)~~, 4 hours ~~(Lane 806)~~, 6 hours ~~(Lane 808)~~, 10 hours ~~(Lane 810)~~, and 24 hours, as indicated. ~~(Lane 812)~~. Lane 0 ~~800~~ represents the zero time point; and lane 24W/O ~~Lane 814~~ represents the control where no ethylene was applied. Increased levels of expression of the gene product are observed in the 2 hour, 4 hour, 6 hour, 8 hour, 10 hour and 24 hour treated samples ~~lanes 804, 806, 808, and 812~~, for example. The apparent low level of signal in the 10 hour treated sample ~~Lane 810 at 10 hours~~ may be due to insufficient sample load. The experimental protocol was similar to that in Example 3, except that a HindIII restriction fragment of potato amt gene was used as the probe.